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HEAT DESTRUCTION OF AFRICAN SWINE FEVER VIRUS IN THE PORTUGUESE "CHOURIÇO". TECHNOLOGICAL PARAMETERS.

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SUMMARY

This work shows that the thermal processing used by the Portuguese industry can offer a lethal value which is good enough to insure the safty of this product concerning the African Swine Fever virus. Heat penetration curves on the "critical point" have been determined in several meat plants. Five different technological experiments (chouriço) have been made in a pilot plant with meat and fat from experimentally infected pigs. Different thermal processing has been used. The lethal value has been calculated from the heat processing curves and $F^{4,5}_{60^{\circ}C}$.
Afterwards, survival tests "in vivo" for the virus have been done by intramuscular injection and oral intake.
It has been verified that the samples whose thermal processing reach "the critical point" a lethal value above 14 ($F^{4,5}_{60^{\circ}C}$.) did not produce infection in the test pigs. Below lethal values of 14 ($F^{4,5}_{60^{\circ}C}$.) all test pigs died.

1 - INTRODUCTION

The fresh and processed pork, from countries where the ASF appears, are considered to be important factors of spreading of the disease.
As a rule of sanitary defense against the spreading of ASFV, the circulation of those raw materials and products from countries which have the disease, to countries free of this problem is not allowed. The exception are the sterilized canned products.
According to the presente data on the heat resistance of the ASFV, we feel that thermal treatments above 60°C are strong enough to insure the sanitary safety of processed meat products.
The industrial portuguese "chouriço" is among the meat products which source comes from pork.

2 - OBJECTIVE

To show that the technology of the Portuguese Industry in the production of "chouriço" insures (in a great margin of safety) this product concerning the ASF virus.

3 - KNOWN DATA

3.1 - "chouriço" processing tecnhology

This product is made by the Portuguese industry with pork and fat and has its source in traditional country style food. Basically, the manufacturing process has the following steps:

a) Selection and mechanical grinding of raw materials in pieces greater than 1 cm;
b) Seasoning and blending of all ingredients.
The seasonings used are not always the same, but usually are: salt (sodium chloride), pimiento paste, paprika, garlic and red wine. The chouriço Portuguese standard (N.P. 589/85) allows the addition of the following additives: phosphates (sodium and potasium salts), ascorbic acid and/or its sodium and potasium salts, nitrites and nitrates (sodium and potasium salts). All these additives are allowed to certain limits.
c) Filling up, by a mechanical way, in a natural (pork and cow) or organosynthetic casings with a diameter between 35-40 mm. Each "chouriço" should have a maximum length 40 cm, with a right-lined or horseshoe configuration.
d) Stewing and smoking.
The first technological step's main objective is the product stewing with progressive temperatures and controled humidities, for approximately 4-5 hours in a stewing-smoking house. These temperatures can reach 80°C at the end of the process with an internal product temperature of at least, 65°C. The thermal values used here, are different to each processor.
The second step, the smoking of the product, can be started at the same time of the stewing. Usually, this is made in traditional smoking house at 40-50°C, for 18-24 hours. At this point the dehidration attain a level that will allow the conservation of the product.
e) Packaging - in cans with vegetable oil or under vacuum in a plastic film, or in a retortable pouch (plastic and aluminium).

3.2 - Heat sensitivity of ASF virus

The heat resistance of different strains of the ASF virus is has been studied by some researchers in different substracts (1;2;3;4;5;6;7;8).
From the results obtained by them, we can say that the virus is sensitive to temperature, in the range of 55-60°C and above. It is also shown that the thermal inactivation of the ASF virus S-60 strain, in the supernatant of a meat homogenate from experimentally infected pigs, was done in a logharitmic way.
This behaviour allows us find the thermal resistance parameters of ASFV - the time/temperature relationships represented by the D and Z values (9). A table of lethal values for this strain and based on these parameters, appears in the literature (F values for Z=4,5°C and reference temperature T=60°C) for temperatures between 50-75°C (10). With these values the efficiency of the thermal treatment used in the processing of meat products, in relation to this virus strain, can be found.

4 - EXPERIMENTAL WORK

Five experimental productions of "chouriço" were made in the pilot plant with meat and fat from experimentaly infected pigs with ASFV S-60 strain (one pig for each experiment), according to the technological process used by the Portuguese industry. The titer of the ASFV in the meat was determined for each experiment.
Experiments 1,2 and 3 were submitted to the same heat treatment in the same stewing operation. Experiments 4 and 5 were submitted to the different thermal treatments. This way we try to inactivate the ASFV by different internal temperatures of "chouriço" and time of processing.

From each experiment we took many samples. One was taken before the starting of the stewing to confirm the existence of the active virus form.
Other samples were taken during the stewing process at certain inside temperatures of the chouriço to verify the survival of the virus.
In every experiment the evolution of the stew temperature and the heat penetration curve in critical point of the "chouriço" during the process was recorded. The lethal value of the heat penetration curve of every sample was analized until they were taken out of the stew. The heat penetration curves during the stewing, made by industrial processors was recorded and its lethal value was determined.

5 - MATERIALS AND METHODS

a) infected meat

1) **Virus** - ASFV 60 Lisbon Strain
2) **ASFV-infected meat** - Susceptible large-white pigs were innoculated by intravenous with 2 ml of a stock virus suspension (mixture of blood and spleen) and highly virulent ASFV-strain (1960) containing aproximately 1.000.000TCIDF 50/ML. Three days after inoculation virus multiplication in the pigs was monitored by Elisa Test with favourable results. The pigs were slaughtered on the fourth day post-inoculation.
3) **Meat preparation** - Pig meat for technological work was a mixture of muscle from legs and shoul ders with the lymph nodes. Pork fat was added and the mixture was stored at 0-5°C for 24 hours.
4) **Detection of ASFV** - Each sample of 3 "chouriços" about 450g, was divided into two equal portions. One of the portions was homogenized with 300 ml of saline solution in a waring blender for 3 mn. This homogenate, after being frozen at - 70°C overnight was thawed and passed through 3 fold gauze and centrifuged at 4.000 rpm for 20 mn. The supernatant (about 100 ml) was inoculated in susceptible pigs by subcutaneous route. The other portions of the sample were administered orally.

b) Process Technology

The technological process was refered to in point 3.1. We used the following seasonings and additives: 3,6% of pimiento paste; 0,25% of paprika; 0,025% of garlic; 2,5%of salt; 0,3 of polyphosphate mixture. The filling of meat paste was in organic-synthetic casing "Fibran", 36mm in diameter. The "chouriço" was individualized with 20 cm of length. The stewing was made in a stew-smoke house "voss 800" equiped with humidity and temperature recording and control. The smoking of the product was made in a traditional smoke house at 40-45°C.

c) Recording Temperature Equipment

We use the Ellab Termograph recorder with needle thermocouples to record internal temperature of the "chouriço" and thermocouples TC-67 to record the stew-smoke house temperature.

d) Lethal Value Calculation

The lethal value calculations were made based on the heat penetration curves inside the chouriço. We used the method of PATASHNIK (11) and the table of lethal values reported to a $F^{4,5}_{60^oC}$ (table 1) as suming that the lethal values of temperatures inside the chouriço were above 50°C during the process.

6 - RESULTS AND DISCUSSION

Virus assays during the meat preparation until the flavouring were carried out in bone marrow cell cultures. The titers were calculated by Karber's method. The virus titers were:

1 st experiment - $10^{5,16}$
2 nd experiment - $10^{4,16}$
3 rd experiment - $10^{4,83}$
4 th experiment - $10^{5,3}$
5 th experiment - $10^{4,6}$

The temperature evolution on the "chouriço's" coldest point, observed during the different thermal processes is recorded on Fig.1 and Tables 2,3 and 4, only for temperatures above 50°C.
As we can see, the evolution of the critical temperature related to time is different for each experiment.
Consequently, we have different times for the same inside temperature in different thermal processing.
The time, critical temperature and lethal value of the thermal processing when the samples were taken for survival tests of the ASFV are recorded in Table 6.
As we wanted to prove, the samples taken at the same temperature at the critical point showed different survival values (table 6 - samples 2 and 3). It can also be observed, that the sample nº 2 in 5 th experiment taken at lower internal temperature than samples nº 1 from the experiments 1, 2, 3 and 4, showing a negative result whereas the others presented positive results.
These two findings, prove that the ASFV S60 heat resistance depends on the temperature and time of the thermal processing, as it has been said by several authors. The results are recorded in lethal values column of Table nº5. These values mean that the intensity of the thermal processing ralated to the ASFV heat resistance, and they are dependent of the temperature evolution on the coldest point, during the thermal processing
From these we can note negative results for lethal values ($F^{4,5}_{60^oC}$) above 14,028 for the virus survival tests independently of the internal temperature observed in the "chouriço".
In the three experiments processed at the same time with the same heat treatment, only one gave positive results in the survival tests. This is acceptable, because the resistance of microorganisms to heat is a function of several factors, namely the chemical composition of the product.
Table 5 shows of the heat treatment used by some industries in the production of "chouriço".
All are different, but all of them have lethal values ($F^{4,5}_{60^oC}$) much higher than those necessary to obtain the inactivation of the ASFV in our tests.

7 - CONCLUSIONS

From the results obtained in the experiments and taking into account what we said in the previous discussion we can conclude:

1 - That the thermal processing used by the Portuguese meat industry in the production of "chouriço", has sufficient strength to assure the of safety this product in relation to the ASFV.
2 - That the efficiency evaluation of the thermal processing in relation to the ASFV must be calculated by the lethal values instead of temperatures in the coldest point of the product or the time of processing. For the S.60 strain the lethal values must be found in order to a Z=4,5 and reported to 60°C

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Fig. 1: Heat Penetration curves in the coldest point of "chouriço" at the thermal processing for the study of the survival of ASFV.

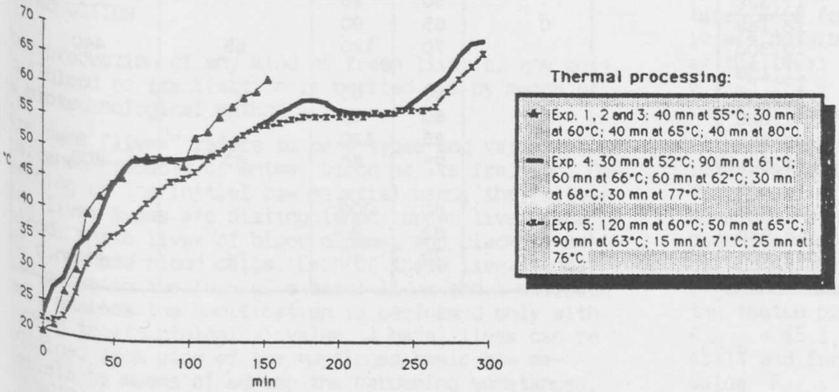

TABLE 1 (10)

Lethal values $10^{\frac{T'-T}{Z}}$ for T=60°C and Z=4,5°C

T°C	$F^{4,5}_{60°C}$	T°C	$F^{4,5}_{60°C}$
50	0,006	63	4,642
50,5	0,008	63,5	5,995
51	0,010	64	7,743
51,5	0,013	64,5	10,000
52	0,017	65	12,915
52,5	0,022	65,5	16,680
53	0,028	66	21,543
53,5	0,036	66,5	27,823
54	0,046	67	35,942
54,5	0,060	67,5	46,420
55	0,077	68	59,951
55,5	0,100	68,5	77,428
56	0,129	69	100,000
56,5	0,167	69,5	129,152
57	0,215	70	166,802
57,5	0,278	70,5	215,424
58	0,359	71	278,227
58,5	0,464	71,5	359,335
59	0,600	72	464,194
59,5	0,774	72,5	599,515
60,	1,000	73	774,273
60,5	1,292	73,5	1000,000
61	1,668	74	1291,500
61,5	2,154	74,5	1668,000
62	2,782	75	2154,200
62,5	3,592		

TABLE 2

Temperature evolution in the critical point of "Chouriço"
Exp. 1, 2 e 3

Time mn	T°C	$F^{4,5}_{60°C}$
0	20	-
		-
96	48	-
100	50	0,024
104	52,5	0,112
108	53	0,224
112	53,5	0,368
116	54,5	0,608
120	56	1,124
124	57	1,984
128	57,5	3,096
132	58	4,532
136	59	6,932
140	59,5	10,028
144	60	14,028
148	61,5	22,644
152	62	33,772

TABLE 3

Temperature evolution in the critical point of "chouriço"
Experiment 4

Time mn	T°C	$F^{4,5}_{60°C}$	Time mn	T°C	$F^{4,5}_{60°C}$
0	27	-	208	56,5	18,228
			212	56,5	18,996
120	50	-	216	56,5	19,664
124	51	0,064	220	56,5	20,332
128	51,5	0,116	224	56	20,848
132	52,5	0,204	228	56	21,364
136	53	0,316	232	56	21,880
140	53,5	0,460	236	56,5	22,548
144	54	0,644	240	56,5	23,216
148	54,5	0,884	244	57	24,076
152	55	1,192	248	58	25,512
156	56	1,708	252	59	27,912
160	56,5	2,376	256	59,5	31,008
164	57	3,236	260	60	35,008
168	57,5	4,348	264	61	41,680
172	58	5,784	268	61,5	50,296
176	58,5	7,640	272	62,5	64,664
180	59	10,040	276	64	95,636
184	58,5	11,856	280	65,5	162,356
188	58	13,292	284	66,5	229,076
192	58	14,728	288	67,5	414,756
196	57,5	15,840	292	68	654,560
200	57	16,700	296	68	884,364
204	57	17,560	300	68	1124,168

TABLE 4

Temperature evolution in the critical point of "Chouriço"
Experiment 5

Time mn	T°C	$F^{4,5}_{60^oC}$	Time mn	T°C	$F^{4,5}_{60^oC}$
0	22	-	208	56	7,616
			212	56	8,132
120	50	-	216	56	8,648
124	50,5	0,056	220	56	9,164
128	51,5	0,108	224	56,5	10,832
132	52	0,176	228	56,5	11,500
136	53	0,288	232	56,5	12,168
140	53,5	0,432	236	57	13,028
144	54	0,616	240	57	13,888
148	54	0,800	244	57	14,748
152	54,5	1,040	248	57	15,608
156	55	1,348	252	57	16,468
160	55	1,656	256	57	17,328
164	55,5	2,056	260	57	18,188
168	55,5	2,456	264	57,5	19,300
172	56	2,972	268	58,5	21,156
176	56	3,488	272	60	25,156
180	56	4,004	276	61	31,828
184	56	4,520	280	62	42,956
188	56	5,036	284	63	61,524
192	56	5,552	288	64	92,496
196	56	6,068	292	64,5	132,496
200	56	6,584	296	66	218,668
204	56	7,100	300	67	362,436

TABLE 5

Thermal Process for "Chouriço" used in the portuguese industry

Factory	Thermal treatment T°C	Thermal treatment time mn	Critical point at the end of the process	Lethal values $F^{4,5}_{60^oC}$
A	55	60		
	60	30		
	70	40		
	75	110	66	829
B	57	40		
	60	20		
	65	60		
	76	100	66,5	561
C	50	90		
	65	90		
	70	120	65	440
D	50	60		
	60	60		
	65	120		
	75	60	67	808
E	60	200		
	62	20		
	70	40	64	214

TABLE 6

Survival of ASFV in "Chouriço" during the thermal process.
Highest temperature reached and lethal value corresponding to each sample

Sample Nº	Experiment Nº	Proc. Time mn	Highest temperature reached in the critical point: Temperature T_1 (°C)	Highest temperature reached in the critical point: Time at Temperat T_1 mn	Lethal values ($F^{4,5}_{60^oC}$)	in vivo test 1 pig for sample
0*	1	--	--	--	--	Pig died with acute ASF
	2	--	--	--	--	" " " "
	3	--	--	--	--	" " " "
	4	--	--	--	--	" " " "
	5	--	--	--	--	" " " "
1	1	124	57	1	1,984	Pig died with acute ASF
	2	124	57	1	1,984	" " " "
	3	124	57	1	1,984	" " " "
	4	164	57	1	3,236	" " " "
	5	172	56	1	2,972	" " " "
2	1	144	60	1	14,028	Pig died with acute ASF
	2	144	60	1	14,028	Survival pigs, one for
	3	144	60	1	14,028	samples, 21 days post
	4	240	57/59	76	23,216	administration sample.
	5	256	56/57	84	17,328	At this time samples
3	1	152	62	1	33,772	for ASFV antibodies
	2	152	62	1	33,772	were negative. After
	3	152	62	1	33,772	that time all pigs
	4	260	60	1	35,008	were chellenged until
	5	272	60	1	25,156	the ASFV 60.
4	4	300	68	1	1.124,168	All pigs died from acute ASF
	5	300	67	1	362,436	

* samples of "chouriço" were taken before thermal processing